Advanced Journal of Microbiology Research Volume 2013
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Microbial solubilization of Ogun rock phosphate in the laboratory and in soil

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Accepted 28 October 2012

Biological solubilization of rock phosphate is more environmentally friendly than acidulation. There is a need therefore to develop a microbial process that will make phosphorus available for plant use with minimum pollution to the environment. Solubilization of phosphorus (P) from Ogun rock phosphate (ORP) in a defined growth medium and in soil was carried out to evaluate the potential of a mixed culture of soybean and cowpea rhizobia (IRj 284 and IRc 252, respectively), as well as a mixed culture of *Bacillus subtilis* MS10 and *Aspergillus niger* Tiegh for use in such a process. In broth medium, the amount of P released increased as the rhizobial inoculum size increased. An increase in microbial population correlated positively (r^2 = 0.72) with P release in the broth at day 1. In the soil, however, the P release peaked at 10 days after inoculation. In broth medium, a mixed culture of *B. subtilis* and *A. niger* increased P release earlier in the solubilization period compared to that obtained with cowpea and soybean rhizobia. There was also a positive correlation (r^2 = 0.58) between an increase in the microbial population and P release a day after inoculation. In contrast, the microbial population was poorly correlated with P release a day after inoculation in soil. However, a stronger correlation was observed at day 5 and 10 with r^2 values of 0.309 and 0.420, respectively, compared to the initial r^2 value of 0.009 at day 1. Nevertheless, efficient ORP solubilization occurred in the soil between the 5th and 15th day after microbial inoculation. It is expected that legumes may benefit from this process, because this period marks the beginning of nodule initiation, nodule formation and nitrogen fixation.

Key words: Ogun rock phosphate, solubilization, *Bradyrhizobium*, *Aspergillus niger*, *Bacillus subtilis*, soil.

INTRODUCTION

Acidulation, using mineral acid or through a biological method, will yield phosphate but in different quantities, depending on the environmental factors. Although acidulation using mineral acid is faster, microbial solubilization is a natural process and is environmentally friendly. Paulraj and Velayudham (1995) investigated the effect of using Mussorie rock phosphate (MRP), organic manure and phosphate- solubilizing bacteria on the yield of rice. They found that the rice yield was highest when a single super phosphate (SSP) was used, but the yield did not differ significantly from that obtained when MRP that was enriched with both the manure and bacteria, was used as fertilizer. Singh and Kapoor (1998) assessed the effect on *Vigna radiate* following inoculation of phosphorus-deficient natural sandy soil with MRP in the Presence or absence of phosphate-solubilizing bacteria (*Bacillus circulans* and *Cladosporium herbarum*) and an Arbuscular fungus, *Glomus fasciculatum*. The results indicated improved nitrogen (N) and phosphorus (P) uptake after treatment with a combination of *B. circulans, C. herbarum* and *G. fasciculatum* in the presence of MRP. Furthermore, Manjaiah et al. (1996) reported that the pod yield of groundnut was higher when MRP and farm yard manure were mixed with *Aspergillus awamori* compared with the use of a single super phosphate (SSP) and MRP alone. The authors also studied the influence of MRP and organic amendments, with or without a phosphate-solubilizing bacterium (*Pseudomonas straita*) and fungus (*A. awamori*) on the nutrient status of an acidic sandy soil. They found that a combination of MRP, organic amendments and phosphate-solubilizing microorganisms made N, P, K and S available.

Preliminary studies have indicated that *Ogun* rock phosphate (ORP) enhances plant growth and crop yield. Different application methods such as direct application or pre-application acidulation gave varying results. There is

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Table 1. Physico-chemical analysis of the soil used in this study.

Parameters	Values
Sand	82.0%
Silt	12.0%
Clay	6.0%
N	0.11%
C	1.28%
Ca (meq 100^{-1})	2.60
Mg (meq 100^{-1})	1.73
K (meq 100^{-1})	0.21
Na (meq 100^{-1})	0.66
H^{+} (meq 100^{-1})	0.11
Cation Exchange Capacity (CEC)(cmol/kg)	5.31
Base	98%
Average P	1.46 mg/kg
pH	5.9
Microbial population	7.2 x 10^{8} CFU/g

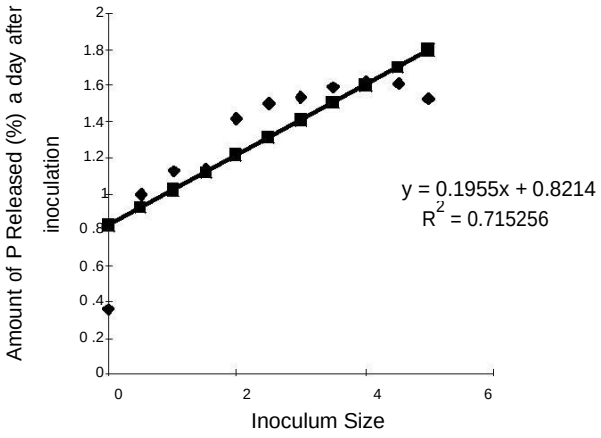

Figure 1. Amount of phosphorus (P) released (%) as influenced by varying sizes of the rhizobial inoculum.

is, therefore, a need to evaluate the phosphorus yield *in vitro* and within the soil environment in order to adopt a better approach in ORP solubilization for enhanced crop production. This study examined microbial solubilization of ORP prior to its application to soil, as well as *in situ* in soil by selected microorganisms.

MATERIAL AND METHODS

Laboratory studies with mixed cultures of microorganisms

In the first laboratory study, *Bradyrhizobium japonicum* IRj 284 and a cowpea rhizobium IRc 252 were used as mixed culture to solubilize ORP. Ammonium sulphate-yeast extract-glucose (AYG) medium (Halder et al., 19990) was used with ORP powder (~240 mesh size) as a source of insoluble phosphate. The medium contained: glucose, 20.0 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; yeast extract, 0.2 g; $FeCl_3$, 2.0 mg; $MnSO_4.H_2O$, 4.0 mg in 1 lire of distilled water (Halder et al., 1990). The pH of the medium was adjusted to 6.8 and volumes of 100 ml were dispensed into a number of 200 ml flasks. Following autoclaving at 121^{o}C for 15 min, the flasks were each inoculated with the individual isolates (0 -5%) and incubated at 28^{o}C on a rotary shaker. Three flasks were left uninoculated and used as controls. Samples were taken at 1, 5, 10, 15 and 20 days after inoculation. Subsequently, the culture medium was centrifuged at 15 000 x *g* for 20 min to remove the biomass and un-solubilized matter. The P content was determined using a Spectro-20 electrophotometer according to the procedure described by Jackson (1958). In the second laboratory study, performed as described above, a mixed culture of *Bacillus subtilis* and *Apergillus niger* was used to solubilize ORP. The broth used in this second study was carefully formulated to accommodate the nutritional requirements of both the bacterium and fungus, and ORP was again used as the main source of P. Thus, 2 g of yeast extract and 5 g each of peptone and NaCl were combined with 4 g of potato extract and 20 g of dextrose, and dissolved in 1 litre of distilled water. All studies were performed in triplicate.

In situ soil studies with mixed cultures of microorganisms

Soil samples used in this study were initially characterized regarding their physico-chemical properties and the total viable count was determined (Table 1). Mixed cultures of the rhizobia or of *B. subtilis* and *A. niger* were mixed with ORP before their application (0 to 20%) to 1 kg of soil, and sampling was performed at 1, 5, 10, 15 and 20 days after inoculation. All of the studies were performed in triplicate.

Data analyses

All data generated were subjected to regression analysis using the SAS statistical package.

RESULTS AND DISCUSSION

Microorganisms bring about a number of transformations of the chemical elements in soil, including the oxidation or reduction of inorganic phosphorus (P) compounds. In this process, P that is in excess to the required amount is released into the soil environment. The efficiency of the process depends, amongst other, on the population of the organisms and environmental factors. In this study, as shown in Figure 1, the population of rhizobial cells was positively and strongly correlated (r^2 = 0.72) with the amount (%) of P released into the growth medium at the first day after inoculation. However, at the 15th day after inoculation, the correlation (r^2 = 0.45) was not as strong as that observed in the first day (Figure 2), indicating that as the cells aged, there was a reduction in the effectiveness of the bacterial population to effect P release from the rock substrate. This was also evident in Figure 3 where a reduction of P released into the medium was observed, despite an increase in the inoculum size. This may be an indication that a considerable amount of P was immobilized by the bacteria.

In our soil inoculation study, there was a weak correlation (r^2 = 0.02) between the rhizobial population and the amount of P released into the soil in the first day of inoculation (Figure 4) . The weak correlation was expected, because the soil P was low and P released at the first day by microbial action would have been fixed by both clay mineral and organic compounds (humic and fu-

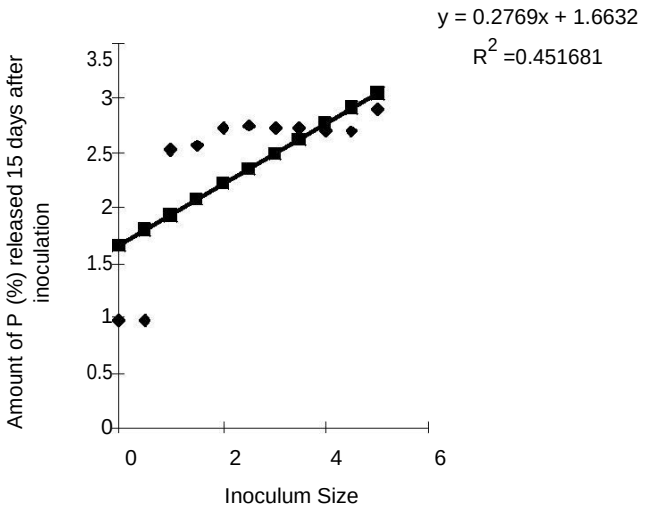

Figure 2. Amount of phosphorus (P) released (%) as influenced by varying sizes of rhizobial inoculum.

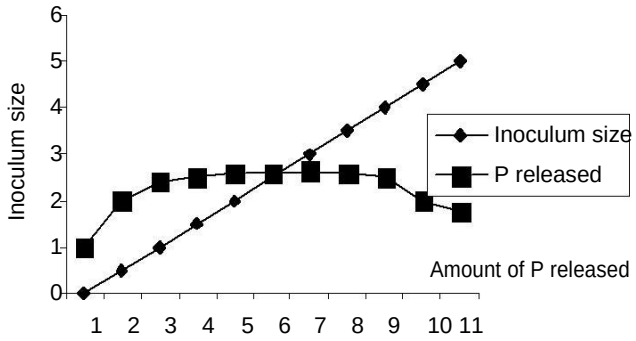

Figure 3. Phosphorus (P) in broth (%) in response to increase in inoculum size of rhizobium.

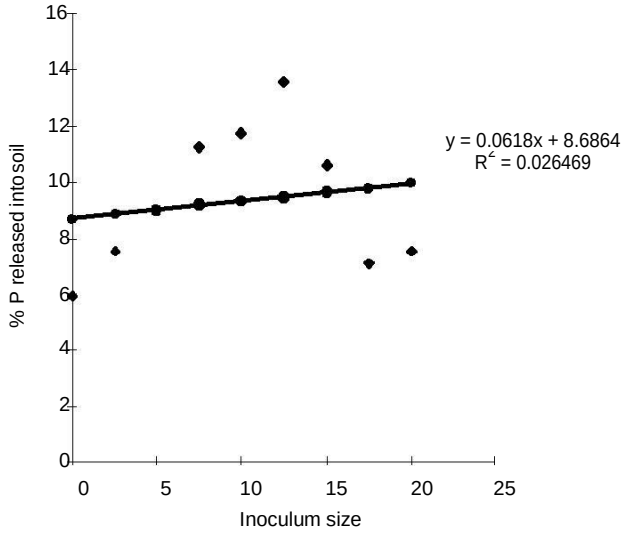

Figure 4. Amount of P released into broth from *Ogun* RP, a day after rhizobial inoculation.

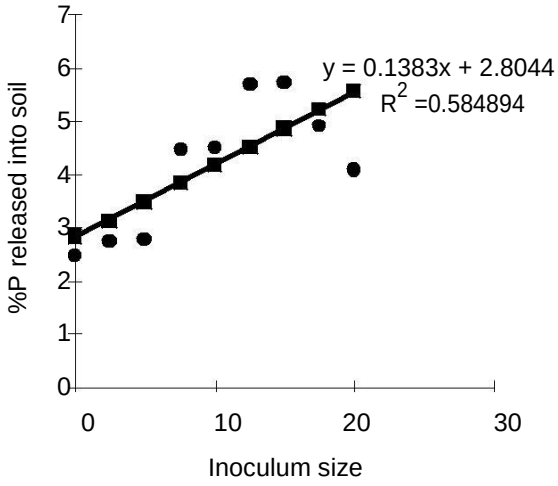

Figure 5. Amount of P released into soil (%) from *Ogun* RP on the 10th day as influenced by rhizobial inoculation.

vic acids) in the soil, as well as by microbial cells. However, P release peaked at the 10th day after inoculation and there was a strong positive correlation (r^2 = 0.58) between the bacterial population and P release (Figure 5). Since the initial P value of the soil used in this study was low (1.46 mg/kg) (Table 1), it would suggest that a considerable amount of P is required to meet the soil biological P demand. Some leguminous crops such as cowpea (*Vigna unguiculata*) and soybean (*Glycine max*) may therefore benefit from the P flux, since that period marks the beginning of nodule formation and symbiotic nitrogen fixation.

In broth inoculated with both *B. subtilis* and *A. niger*, there was a positive correlation (r^2 = 0.58) between an increase in the inoculum size of the mixed culture and the amount of P released into the medium at the first day after inoculation (Figure 6). However, an increase in the inoculum size did not significantly increase P released even at 15 days after inoculation (Figure 7), suggesting that there was microbial immobilization of P during this period of time. Several reasons may be ascribed to this observation. The first is that a reduction in nutrients in the growth medium might have altered the P availability. Alternatively, a reduction in the activities of viable cells might have occurred or the aging cells might be unable to effectively carry out their biochemical functions of P mineralization. Sahram (1999) reported a relationship between the rate of solubilization of rock phosphate and extracellular exudates, including organic acids, as well as alkaline and acid phosphatases in the growth medium. It should be noted that solubilization of ORP with a mixed culture of *B. subtilis* and *A. niger* might not be a rational option for producing P from ORP, especially if the P re-

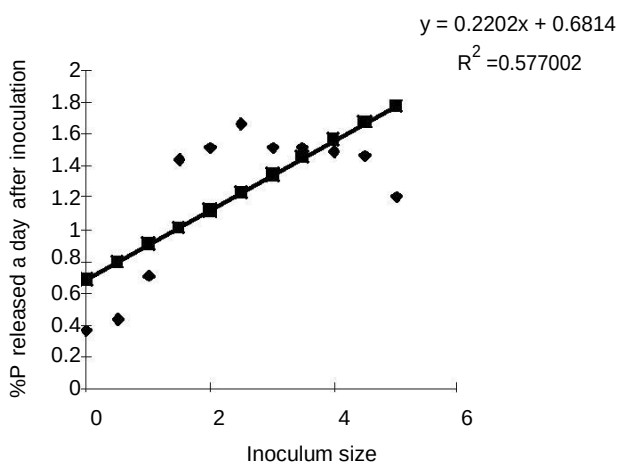

Figure 6. Amount of P released into broth from *Ogun* RP as influenced by mixed culture of *Bacillus subtilis* MS 10 and *Aspergillus niger.*

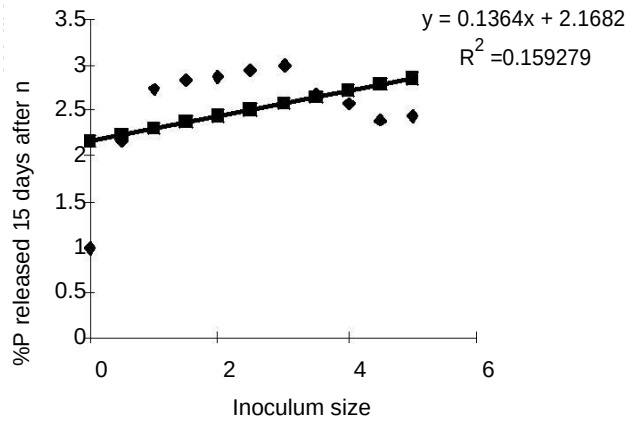

Figure 7. Amount of P released into broth from *Ogun* RP as influenced by mixed culture of *Bacillus subtilis* MS 10 and *Aspergillus niger.*

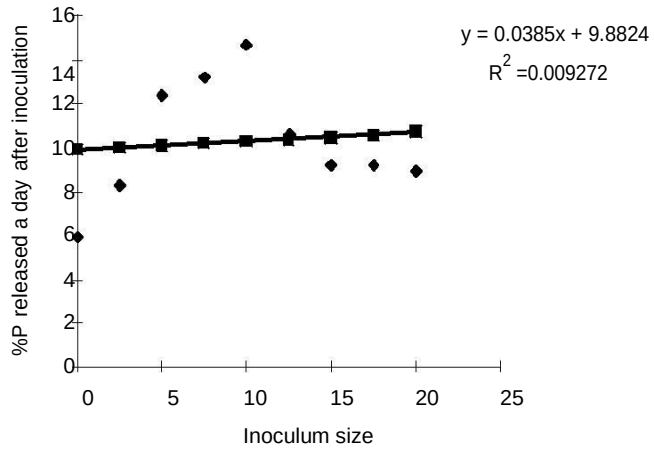

Figure 8. Amount of P released into broth from *Ogun* RP as influenced by mixed culture of *Bacillus subtilis* MS 10 and *Aspergillus niger.*

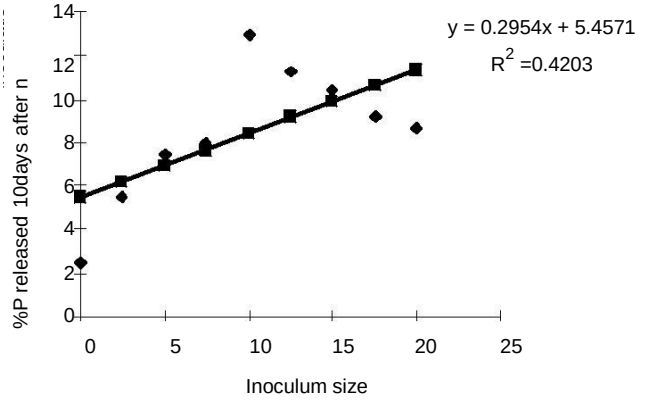

Figure 9. Amount of P released into broth from *Ogun* RP as influenced by mixed culture of *Bacillus subtilis* MS 10 and *Aspergillus niger.*

released was to be used by growing plants. Nevertheless, the bulk-soil microbes do impact markedly and positively on P release either from the added rock phosphate or from the less available indigenous P sources, especially when there are sufficient amounts of nutrients present in the growth medium. This is corroborated by a report of Toro et al. (1997) who found that phosphate-solubilizing rhizobacteria, *Enterobacter* spp. and *B. subtilis* influenced the biochemical cycling of P in a positive way.

In soil, the *B. subtilis* and *A. niger* population correlated poorly (r^2 = 0.01) with the amount of P released from ORP a day after inoculation (Figure 8), but it improved at 10 days after inoculation (Figure 9). This is similar to the results obtained when rhizobia were used as soil inoculant. The results indicated that the benefits of microbial solubilization would, most of the times, become feasible from the 5th and the 10th day after inoculation, especially in soil. Also, microbial solubilization of rock phosphate appeared to be dependent on the time of inoculation and application to soil. Late application in the crop life cycle may not be of considerable benefit to the crops, albeit that P release from rock phosphate is generally enhanced by the microbes. The reports of Burford et al. (2003), Didiek et al. (2000), Taiwo and Oso (1997), and Gull et al. (2000) are consistent with this assertion. Reyes (2002) similarly obtained an increase in maize yield when a bacterium and *Penicillum rugulosum* strain were used with rock phosphate.

Conclusion

In this study, there was a general increase in P solubi-

lized from ORP by all the microorganisms (*Rhizobium*, *Bacillus* sp. and *A. niger*) . However, correlation between microbial population and P released weakened as the microbial cells aged. *Ogun* rock phosphate solubilization was good between the 5th and 15th day after inoculation. Solubilization of ORP in the soil, using *B. subtilis* and *A. niger* , may be a option of P production from ORP, especially before the 20th day of introduction to the soil.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Dauda, O. R., Adegoke, O. A. and Durojaye, O. of Ibadan Polytechnic for their assistance in the incubation experiment and laboratory analysis of all the samples. We also thank the management of Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan for allowing publication of this work.

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